QTL ANALYSIS OF PEST RESISTANCE IN THE WILD TOMATO, *LYCOPERSICON PENNELLII*: QTL CONTROLLING ACYLSUGAR LEVEL AND COMPOSITION

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Abstract Some accessions of *Lycopersicon pennellii*, a wild relative of the tomato, *Lycopersicon esculentum*, are resistant to a number of important pests of cultivated tomato due to the accumulation of acylsugars, which constitute 90% of the exudate of type IV trichomes in *L. pennellii* LA716. An interspecific F_2 population created by the cross *L. esculentum* x *L. pennellii* LA 716 was surveyed for acylsugar accumulation and subjected to RFLP analysis to determine the genomic regions associated with the accumulation of acylglucoses, acylsucroses, and total acylsugars, and with acylglucoses as a percentage of total acylsugars (mole percent acylglucoses). Data were analyzed using MAPMAKER/QTL with and without $\log_{10}$ transformation. A threshold value of 2.4 (default value for MAPMAKER/QTL) was used, as well as 95% empirically derived threshold values. Five genomic regions, two on chromosome 2 and one each on chromosomes 3, 4, and 11, were detected as being associated with one or more aspects of acylsugar production. In regions on chromosome 2 and 11 the *L. esculentum* allele is partially dominant to the *L. pennellii* allele, but the *L. pennellii* allele is dominant in the region on chromosome 3. Throughout this study, we comparatively report the effects of analytical methodology on identification of acylsugar QTL. Similarities between our results and published results for the genus *Solanum* are also discussed.

Key Words: Acylsugars, pest resistance, QTL analysis, tomato, RFLP

Introduction

Cultivated crops are attacked by a large number of insect pests which damage plants and reduce yields. Pests are chiefly controlled by the use of synthetic pesticides, which is an expensive process both in the production of the pesticides and in their repeated applications over the growing season. Use of pesticides is increasingly limited by evolution of pesticide resistant insects, withdrawal of chemicals due to loss of label, and increasing health/environmental concerns. The incorporation of resistance into crop species from their wild relatives provides an alternative method of pest control.

Plant trichomes are a key defense mechanism against herbivorous arthropods (Levin 1973; Webster 1975). Glandular trichomes are specialized organs which produce a multitude of secondary plant metabolites which can deter, entrap, or are toxic to arthropods. *Lycopersicon pennellii* (Corr.) D'Arcy accession LA716, a wild relative of the cultivated tomato *L. esculentum* (Mill.), is resistant to several insect species, including greenhouse whitefly (*Trialeurodes vaporariorum*), carmine and two-spotted spider mites (*Tetranychus cinnabarinus* and *T. urticae*), and potato and green peach aphids (*Macrosiphum euphorbiae* and *Myzus persicae*) (DePonte et al. 1975; Gentile and Stoner 1968; Gentile et al. 1968, 1969). The multiple pest resistance of *L. pennellii*, LA716 is mediated by acylsugars exuded by type IV glandular trichomes on the surface of virtually all the aerial portions of these plants. The acylsugars act as feeding deterrents for potato aphid, green peach aphid, tomato fruitworm (*Helicoverpa zea*, formerly *Heliothis zea*), and beet armyworm (*Spodoptera exigua*), and as feeding and oviposition deterrents for the leafminer (*Liriomyza trifolii*) and silverleaf whitefly strain B (*Bemisia argentifolii* formerly sweetpotato whitefly, *B. tabaci*) (Goffreda et al. 1989; Hawthorne et al. 1992; Rodriguez et al. 1993; Juvik et al. 1993; Liedl et al. 1995). Acylsugars also exert a detrimental effect on larval development of tomato fruitworm, and on both larval development and survival of beet armyworm (Juvik et al. 1993). Acylsugars have been

identified in other members of the genus *Lycopersicon*, and in species of other Solanaceous genera, including *Solanum*, *Nicotiana*, *Petunia* and *Datura* (Schumacher 1970; Severson et al. 1985a; King et al. 1986, 1987, 1988, 1990; King and Calhoun 1988; Shinozaki et al. 1991). Acylsugars may also play a role in insect and disease resistance in some of these other species (Gibson and Valencia 1978; Severson et al. 1985b; Holley et al. 1987; Neal et al. 1990; Kennedy et al. 1992; Cutler et al. 1986; Buta et al. 1993).

The acylsugars of *L. pennellii* LA 716 are a complex chiefly comprised of 2, 3, 4-tri-*O*-acylated glucose esters possessing C_4 to C_{12} fatty acids, which constitutes ca. 90% of *L. pennellii* LA716 type IV trichome exudate (Fobes et al. 1985). There is considerable variation among accessions of *L. pennellii* for level of acylsugars produced, and the type of sugar (glucose or sucrose) and fatty acids incorporated into the acylsugars (Shapiro et al. 1994). Cultivated tomato does not accumulate detectable levels of acylsugars, but *L. esculentum* x *L. pennellii* LA716 F_1 plants accumulated moderate levels of 3', 3, 4-tri-O-acylsucroses, 3', 3, 4, 6-tetra-O-acylsucroses and 2, 3, 4-tri-*O*-acylglucoses (in approximately a 60:40 ratio of acylsucroses to acylglucoses, respectively) (J C Steffens, personal communication). The presence or absence of the type IV glandular trichomes that exude acylsugars is simply inherited and controlled by at least two unlinked genes in crosses between *L. pennellii* LA716 and *L. esculentum* (Lemke and Mutschler 1984).

Breeding for oligogenic (quantitative) traits can be problematic due to difficulties in accurately screening for the trait on a single plant basis, and because few plants in a segregating population possess all of the genes required for trait expression. Nonfecundity and the segregation distortion inherent in many interspecific crosses (Mutschler and Liedl 1994; Liu et al. in press, Zamir and Tadmore 1986) can further impede breeding when the desired trait must be transferred from a wild to a domesticated species. The highly saturated RFLP map of tomato, which possesses over 1000 markers providing resolution of *ca.* 1 to 2 cM (Tanksley et al. 1992), permits the systematic dissection of quantitative traits and further identification and transfer of

chromosomal regions associated with such traits (Paterson et al. 1988; Tanksley 1993; Young and Tanksley 1989).

It is clear that acylsugars play an important role in pest resistance in the Solanaceae. While acylsugars are primarily found in wild species, their transfer to cultivated species promises to contribute greatly to improvement of crop resistance to insect pests, and thus a reduction in the dependence on synthetic chemicals for insect control. Information on the genetic control of acylsugar production and the genomic regions associated with acylsugar production would facilitate the transfer of this trait to cultivated tomato. The purpose of this work is to determine within *Lycopersicon*, the genomic regions associated with the accumulation and composition of acylsugars.

Materials and methods

Plants materials An F_2 population was generated from an *L. esculentum* x *L. pennellii* F1, of which the *L. esculentum* parent was New Yorker^{Lp4} (NY^{Lp4}), a plant derived from the *L. esculentum* line New Yorker but containing the cytoplasm of *L. pennellii* LA716 (Lp) (Mutschler 1990), and the *L. pennellii* parent was *L. pennellii* LA716 (PI246502). Two hundred and thirty four F_2 plants, and the parental and $F_1{}^{Lp4}$ controls, were grown at the Guterman Greenhouse under normal greenhouse conditions.

Acylsugar assays Acylsugars accumulated on control and F_2 plants were surveyed at 16 weeks of age. Three samples were taken per plant to provide replication. The collection of each sample involved rinsing 2 to 3 leaflets in 10 ml of methylene chloride in a scintillation vial. The leaflets were saved and their surface area of assayed leaflets was measured with a LI-COR model LI 3000 area meter to allow consideration of acylsugar accumulation per unit area. The rinsates were backwashed with water to remove free sugars, dried, then resuspended in 2 ml of ethanol. The acylsugar content of the samples were then measured by a modified Nelson's assay for reducing sugars, as

described by Goffreda et al. (1990). The acylsugar levels of each sample were assayed with and without prior treatment with invertase, to allow estimation of acylsucroses as well as acylglucoses. The traits directly measured by the assay are the accumulation of acylglucoses and total acylsugars. These data are used to calculate the accumulation of acylsucroses and acylglucoses as a percentage of total acylsugars (mole percent acylglucoses). Since the molecular weights of acylsucroses and acylglucoses are considerably different, measurements of the accumulation of acylsucroses and acylglucoses were considered in nmol/cm^2 leaflet area, to provide unbiased estimation of mole percent acylglucoses. Since samples were collected/analyzed in triplicate, the values used for each of the acylsugar traits is the average of the results from the three samples obtained per plant.

Mapping: Genomic DNA was extracted from the F_2 plants, each of the parents, and the interspecific F_1 using the method of Doyle and Dickson (1987). After all the acylsugar samples and a portion of the DNA samples were collected, a mechanical failure in the greenhouse flooded one portion of the house, killing the F_2 plants in that section. Consequently, sufficient DNA for complete RFLP analysis was available for the surviving 144 plants, but limited quantities of DNA were available for the portion of the F_2 population that was killed. Therefore, the initial linkage map was created and acylsugar-related regions were identified using the subset of 144 F_2 plants. DNA of these 144 plants and an additional 52 plants from the F_2 population (total of 196 F_2 plants) was used for fine mapping the regions for which the initial analysis indicated an association with acylsugar production. Approximately 20µg of DNA from each plant were digested with restriction enzymes (BstNI, DraI, EcoRI, EcoRV, HaeIII, HindIII and XbaI), following the manufacturer's suggested conditions, run on 0.8% agarose gels at 20V overnight or 90V for 4-5 hours (Maniatis et al. 1982), and blotted onto GeneScreen

Plus membrane (NEN, DuPont) or Hybond^{TM}-N^{+} membrane (Amersham) as suggested by the manufacturers.

One hundred and fifty mapping clones of known chromosomal locations (supplied by Dr. S D Tanksley), chosen to give markers covering almost the entire genome, were used to create a map of the F_2 populations using MAPMAKER/EXP (Lander et al. 1987). Insert DNAs were labeled using random priming (Feinberg and Vogelstein 1983, 1984). Southern analysis was performed essentially as in Maniatis et al. (1982). Ambiguous genotypes were treated as missing data. An error detection function of the computer software MAPMAKER/EXP v.3.0 (Lincoln and Lander 1992; Lincoln et al. 1992) was used to identify candidates for further typing examination and correction.

Genome composition: The computer program HYPERGENE v.0.9 (Young and Tanksley 1989) was used to estimate the percentage of total genome comprised of *L. esculentum* genes. Percentages of total genome that were homozygous for *L. esculentum* (EE), heterozygous for *L. esculentum* and *L. pennellii* (EP), and homozygous for *L. pennellii* (PP) were also estimated for each F_2 plant. These estimates were based on the genotypes of the RFLP markers and the centimorgan distances between these markers obtained from the linkage analysis. Frequency distribution of the percent *L. esculentum* genome composition and the percentages of EE, EP, and PP in the genome were plotted. The skewness and kurtosis statistics of each frequency distribution were calculated by macro functions of the computer software Microsoft Excel version 4.0 (Microsoft Corporation 1992) to characterize the frequency distributions. The difference between population means of percent genome composition was tested by two-tailed *t*-tests without pooling the sample variances (MINITAB 1991). The difference between the mean of percent genome composition and its expected value was tested by two-tailed *t*-tests.

Linkage map analysis: Genetic linkage maps were constructed based on the monogenic segregation data of RFLP markers, using the computer program MAPMAKER/EXP v.3.0 (Lander et al. 1987; Lincoln et al. 1992) on a Sun Spark 2.0 workstation. All pairs of linked markers were first identified by the 'group' command. Loci orders within linkage groups were verified by the 'ripple' command after initial loci order was achieved through the 'order' command. Distances between markers were estimated by the method of maximum likelihood multi-point analysis. The chromosomal affiliation of each linkage group was established by identifying chromosomal positions of the markers from the published tomato linkage map (Tanksley et al. 1992). All comparisons of map lengths were expressed in Haldane centimorgans (Haldane 1919).

Interval mapping analysis: Chromosomal locations of the putative QTL controlling acylsugar accumulation were analyzed by interval mapping (Lander and Botstein 1989) using the computer program MAPMAKER/QTL v.1.1 (Lincoln et al. 1992). MAPMAKER/QTL assumes that the quantitative data under consideration is normally distributed. However, none of the acylsugar traits being considered fit this assumption. A mixture of normal distributions within each genotypic marker class is expected, rather than normality (Doerge 1993; Doerge and Churchill 1994). When data for a quantitative trait are not normal, the method generally used in QTL mapping papers published to date begins with log transformation of data for normalization. However, it is theoretically not possible to transform a mixture distribution to a single component distribution (Titterington et al. 1985). Furthermore, problems associated within the trait distribution prevents the distribution of the LOD scores from following a function of a standard chi-square distribution (df=1) (Hartigan 1985; Self and Lee 1987). An issue not commonly discussed is that transformation used to normalize data also misrepresents the differences among individuals for the trait by pulling the skewed tails of the distribution toward the center, thus reducing one's ability to detect QTL.

As a point of rising interest in the mapping community, the data for the 144 F_2 plants was analyzed using three comparable methods, and data from the full set of 196 plants was also analyzed by a fourth method :

Method 1 - The distributions of each of the four quantitative traits (acylsucroses, acylglucoses, total acylsugars, and mole percent acylglucoses) were transformed to normality using Log 10 transformation and analyzed using MAPMAKER/QTL, and LOD thresholds of 2.4, which is the default value for LOD thresholds using MAPMAKER/QTL.

Method 2 - The original trait data was analyzed without transformation for the four traits using MAPMAKER/QTL, and LOD thresholds of 2.4.

Method 3 - The original trait data was analyzed for the four traits using MAPMAKER/QTL, and empirically derived threshold values obtained under a permutation setting of 1000 (Churchill and Doerge 1994).

Method 4 - Fine scale mapping was performed for the larger set of 196 F_2 plants for the chromosomal regions in which initial analysis under Methods 1 and 2 indicated QTLs. Chromosomewise 95% (α = 5%) threshold values were calculated using 1000 permutations of the original data (Churchill and Doerge 1994).

For each of these methods, map distances for interval mapping were expressed in Haldane centimorgans (Haldane 1919). QTLs were identified by LOD score calculations at each 2 cM increment across each chromosome (Lander and Botstein 1989; Paterson et al. 1991). Maximum likelihood estimates of both additive (a) and dominance (d) effects were calculated simultaneously during the genome scan for QTLs as performed by MAPMAKER/QTL.

In the process of calculating the thresholds for analysis by Methods 3 and 4, it was found that inclusion of chromosome 10 data impeded this calculation. This was probably due to the extreme skew characteristically found for chromosome 10 in this interspecific cross (Kinzer et al. 1990; Liu et al. in preparation). Therefore, the

thresholds were calculated using data from 11 chromosomes, excluding chromosome 10.

Results and Discussion:

Acylsugar accumulation and composition

L. pennellii LA716 accumulates high levels of acylsugars, the majority of which is composed of acylglucoses (Table 1). In contrast, *L. esculentum* does not accumulate acylsugars, and the low levels of sugar detected represent background levels of the assay. The interspecific F_1 hybrid accumulates levels of acylsucroses similar to that of the *L. pennellii* parent, but less than 10% of the acylglucoses of that parent. As a result, the F_1 hybrid accumulates a much lower total acylsugars level than does the *L. pennellii* parent. In addition, only 50% of the acylsugars of the F_1 hybrid are acylglucoses. compared to 92% in *L. pennellii* LA716.

A wide range of acylsugar accumulation levels is represented in the F_2 (Table 1). Many F_2 plants possess values for levels of acylglucoses, acylsucroses and total acylsugars similar to the background values obtained for the *L. esculentum* parent. Even the highest accumulators of acylglucoses in the F_2 possess far lower levels of acylglucoses than the *L. pennellii* parent. The maximum acylglucoses level for the F_2 is 321% of the maximum acylglucoses level for the F_1, but less than 46% and 36% of the minimum and maximum acylglucoses levels, respectively, for the *L. pennellii* parent. In contrast, the maximum acylsucroses level in the F_2 exceeds that of both the F_1 and the *L. pennellii* parent, at 288% and 274%, respectively. As a result, maximum total acylsugars accumulation in the F_2 population is 235% of that of the F_1, but only approximately 70% and 49%, respectively, of the minimum and maximum total acylsugar levels for the *L. pennellii* parent. Therefore none of the F_2 plants has a total acylsugars level close to that of the *L. pennellii* parent.

Since most of the acylsugars accumulated by *L. pennellii* LA 716 are acylglucoses, but the F_1 only produces *ca.* 50% acylglucoses, it can be deduced that *L. esculentum* contributes gene(s) that affect the mole percent acylglucoses. A surprising feature of the F_2 population is the indication that major gene(s) affect mole percent acylglucoses. The F_2 plants fall into two clusters (plants producing > 60% acylglucoses and <60% acylglucoses, respectively), rather than a random scatter in the plot of total acylsugars versus mole percent acylglucoses (Figure 1). This parallels the observation that *L. pennellii* accessions fall into two classes - those in which acylglucoses represent either approximately 40 or 80% of the total acylsugars produced (Shapiro et al. 1993). Analysis of acylsugar production of the interspecific F_1 indicates that the higher mole percent acylglucoses class is a recessive trait contributed by the *L. pennellii* parent. If one considers only F_2 plants that are clearly acylsugar-accumulating (greater than the lower range of the F_1 control for total acylsugar levels, 100 nmol/cm^2), segregation for high vs. low mole percent acylglucoses (12 to 60 vs. 65 to 95 mole percent acylglucoses, respectively) these data also suggest that the ability to produce the higher mole percent acylglucoses class is a recessive trait contributed by the *L. pennellii* parent. However, a more complete genetic analysis would be required to demonstrate monogenic control.

Genome structure and map of probes:

Although most of the RFLP markers surveyed show the expected codominant segregation for homozygous *L. pennellii* (PP), heterozygous (EP) and homozygous *L. esculentum* (EE) genotypes for the F_2 population, eight of the markers surveyed were of the homozygous *L. pennellii* genotype. The published tomato linkage map (Tanksley et al. 1992) indicates that all of the eight non-segregating markers are located toward the top of chromosome 1 between CT233 and TG71, a region spanning 58.2 cM in the published tomato map (Tanksley et al 1992). The homogeneity of this region for *L. pennellii* alleles in the F_2 population is explained by the fact that the NY^{Lp4} and $F_1{}^{Lp4}$

progenitors of the F_2 population had EP and PP genotypes, respectively, for all of these markers. Therefore, since no recombination can be detected for mapping within this region, it is not included in the analysis of the genome of the F_2 population .

On average, plants in the F_2 population averaged 46.4% *L. esculentum* genome (Figure 2). The mean percentage of *L. esculentum* genome of the F_2 population was significantly smaller than 50% (t = -5.52, df = 143, one-tailed $p < 0.001$). However, the frequency distribution of percent *L. esculentum* genome of the F_2 population was close to the expectation of normal distribution. The proportion of plants having higher than 50% of *L. esculentum* genome was 31.3%. The frequency distribution of genotypes is 19.6% EE genome, 53.6% EP genome, and 26.8% PP genome (Figure 3). The mean percentages of EE, EP, and PP genome of the F_2 population were significantly different from the expectations of 25% (t = -8.12, df = 143, one-tailed $p < 0.001$), 50% (t = 3.67, df = 143, one-tailed $p < 0.001$), and 25% (t = 1.93, df = 143, one-tailed $p = 0.055$), respectively. The heterozygosity of the F_2 population was also higher than expected, and the mean percentage of PP genome was significantly higher than that of EE genome (t = -6.26, df = 256, one-tailed $p < 0.001$). The skewing, which favors the *L. pennellii* alleles and increases the average percentage of the F_2 genome that is *L. pennellii*, is well documented in prior studies of *L. esculentum* X *L. pennellii* F_2 populations (Zamir and Tadmor 1986; Kinzer 1990; Mutschler and Liedl, 1994).

Linkage maps:

Markers included in the map were selected to cover the entire genome with an average distances of about 20 cM between markers. After initial data were analyzed, additional markers were selected from the chromosomal regions showing significant association with total acylsugar levels. Using a LOD score criterion of LOD > 10.0 and MAPMAKER/EXP, the markers separated into 13 linkage groups, since one of the original 12 linkage groups, chromosome 2, was separated into two sublinkage groups,

2-upper, which spans the 8.2 cM between the markers TG31 and TG276, and 2-lower, which spans the 71.9 cM between the markers CT255 and TG141. Markers in region of the split on chromosome 2 showed extreme skewing, which is most likely the cause of the splitting of this chromosome into these sublinkage groups.

The linkage map of the F_2 population contains 150 markers and 137 intervals that cover 1134.8 cM (Haldane cM) with spacing between loci ranging from 0.7 to 29.7 cM, not including the region of chromosome 1 for which the F_2 population does not segregate. Compared with the published tomato linkage map (Tanksley et al. 1992), all but three markers mapped to the same relative positions. Two of the exceptions were cases of reversal of order involving closely linked markers. In each case, the interlocus distance was smaller than 5 cM and one or both of the markers had been mapped with LOD scores < 3.0 in the published map (Tanksley et al. 1992). The third exception, TG28B, mapped individually to a different relative position for its adjacent markers on chromosome 12. The distances between the adjacent markers differed somewhat between our map and the published one, but this is to be expected since the two estimated maps were generated from different populations (sampling effects). Therefore, we consider the differences between the two linkage maps to be insignificant.

QTL mapping:

Five regions, two within the 2-lower portion of chromosome 2 and one each on chromosomes 3, 4, and 11, are detected as being associated with acylsugar production by consensus of the analytical methods used (Table 2). The major effects on both acylsucroses and total acylsugars are associated with the regions identified on chromosomes 2 and 3. The regions on chromosome 2 are associated with acylsucroses by all four of the analytical methods, but cannot be distinguished into two peaks by any of the methods. The regions on chromosome 2 are also associated with total acylsugars by all four of the analytical methods. However, in this case, Method 3 indicates that

only the distal region is associated with total acylsugars, effectively separating the regions. The separation of the effects of chromosome 2 into two distinct peaks is further supported by analysis of mole percent acylglucoses (see below). The proximal region on chromosome 2 has a peak located at or slightly under CD35, and the distal region on chromosome 2 has a peak located at or slightly under TG204. Assuming there are indeed two regions on chromosome 2, their effects on acylsucroses or total acylsugars are remarkably similar. In both regions on chromosome 2, the *L. esculentum* allele is partially dominant to the *L. pennellii* allele, with the heterozygous genotype conditioning slight increases, and homozygous *L. pennellii* genotype conditioning substantial increases, in acylsucroses and total acylsugars. These regions account for a relatively high percentage of the variability observed for these two traits (approximately 11 to 16%, depending on analytical method used). It is possible that the two regions detected on chromosome 2 are derived from an ancestral duplication, although the classical and RFLP maps of tomato (Tanksley and Mutschler 1990; Tanksley et al. 1992) include relatively few indications of duplications on this chromosome. The two regions on chromosome 2 also appear to cause a decrease in mole percent acylglucoses. However, the location of the effects of the distal region on mole percent acylglucoses is not located as tightly to TG204 as are the effects on acylsucroses and total acylsugars. The effects of the two chromosome 2 regions on mole percent acylglucoses is probably indirect, due to the influence of this region on acylsucroses and total acylsugars.

The effects of a region on chromosome 3, with a peak located at or very near TG621, are similar to those of the regions on chromosome 2 in that the *L. pennellii* allele conditions increases in acylsucroses and total acylsugars, as well as decreases in mole percent acylglucoses. However, the *L. pennellii* allele is at least partially dominant to the *L. esculentum* allele in the chromosome 3 region, such that both the heterozygous and homozygous *L. pennellii* genotypes condition a substantial increase in acylsucroses or total acylsugars, as well as a decrease in mole percent acylglucoses. The region

accounts for 7% to 12% of the variability observed for acylsucroses or total acylsugars, depending on the analytical method used, and approximately 6% of the variability observed for mole percent acylglucoses.

The major effect of the chromosome 4 region, with its peak located near TG483, appears to be on acylglucoses, which is associated with the region by Methods 2 and Method 4. The *L. esculentum* allele is either partially dominant or co-dominant to the *L. pennellii* allele, such that heterozygous and homozygous *L. pennellii* genotypes condition moderate and substantial increases, respectively, in acylglucoses. The region accounts for approximately 7% to 9% of the variability observed for acylglucoses, depending on analytical method used. The presence and type of effects due to the chromosome 4 region on other aspects of acylsugar production is less clear. The chromosome 4 region may also have some effect, possibly indirect, on mole percent acylglucoses. However the LOD values for this trait are only slightly over thresholds, and the action of the alleles is not clear as to direction and dominance. Similarly, the chromosome 4 region may have some effect on acylsucroses and total acylsugars. However this effect was only detected by Methods 1 and 4, and the LODs for Method 4 are considerably lower than that for acylglucoses, and there is no agreement as to the direction of the effects and dominance of the *L. pennellii* allele. Again, the effects of the chromosome 4 region on acylsucroses and total acylsugars may be indirect, due to the effect of this region on acylglucoses.

The region on chromosome 11 between TG400 and TG286, with its peak located between these markers and slightly above TG286, is strongly associated with mole percent acylglucoses by all four of the analytical methods. In this region, the *L. pennellii* allele is partially dominant to the *L. esculentum* allele, such that the heterozygous and homozygous *L. pennellii* genotypes condition slight and substantial increases, respectively, in mole percent acylglucoses. This is clearly the strongest of the

associations detected, with LOD scores of 5.2 to 7.2 and the regions accounting for 17.7% to 22.2% of the variability observed, depending on which analytical method was used. The distal peak of the region on chromosome 2 and the region on chromosome 4 were also associated with mole percent acylglucoses by Method 2, and both peaks of the region on chromosome 2 (separately) and the region on chromosome 4 were associated with mole percent acylglucoses by Method 4. However, the effects of the chromosome 2 and 3 regions on mole percent acylglucoses are smaller than those of the chromosome 11 region and/or negative (reducing mole percent acylglucoses), and much lower LOD scores than the LODs of these same regions for acylsucroses and total acylsugar levels. The effects of the chromosome 2 and 3 regions on mole percent acylglucoses could be indirect, through the effects of these regions increasing acylsucroses levels. Therefore the region on chromosome 11 is the only one that appears to fill the expectations for a region in which the homozygous *L. pennellii* genotype conditions distinctly higher levels of mole percent acylglucoses.

Three other regions were identified by at least one of the analytical methods as being associated with acylsugar accumulation. A region at the end of the short arm of chromosome 6 was associated with mole percent acylglucoses by Method 1, and with acylsucroses by Method 2. In addition, a region on the end of the short arm of chromosome 9 was associated with acylsucroses by Method 2. In both of these cases, the regions are very small, are at the ends of the chromosomes, and have LODs just over the thresholds, making it likely that these are not valid QTL and in fact result from the expected 5% false indications of QTL (type I error). A small region at the middle of chromosome 8 was identified only by Method 4 as being associated with acylsucroses, acylglucoses, and total acylsugars. Again, the LODs were just over the thresholds, and the percent variability accounted for by the region ranged from 4.4 to 8.0.

Comparison of analytical techniques

The sole difference between Method 1 and Method 2 is presence or absence of data transformation in an attempt to create a normal distribution of the quantitative trait, as required by MAPMAKER/QTL. Regions of QTL activity identified by MAPMAKER/QTL are effectively the same for Methods 1 and 2, with Method 2 (i.e. untransformed data) providing higher LOD score values and estimates of percentage variability. Method 2 and 3 are equivalent in calculation of LOD scores, but uniquely different in the declaration of significant effects due to QTL activity. The significance threshold used for Method 3 (empirical permutation) are on average higher than the generally accepted 2.4 value. The fact that the threshold values are higher for Method 3 is not surprising; empirical threshold values are derived specifically for the experimental situation at hand, so as to accurately reflect sample size, as well as environmental variation. In general, it appears that the threshold value 2.4 (Method 2) is less conservative than threshold values developed based on the specifics of the data set. Intuitively, this is sensible, since globally accepted threshold values represent "perfect" conditions (i.e. normally distributed data, no skewing, no environmental variation, and no missing data). Realistically, we know that this data is experiencing non-normality, skewing, and missing marker data, as well as biological and environmental effects. In addition, Method 3 accounts for the problem associated with multiple dependent tests. Since we are testing for QTL activity multiple times across a genome of linked genetic markers (137 intervals with increments of 2 cM), the probability of incorrectly declaring a QTL increases. For example, if we make 100 tests for QTL activity using a 5% ($\alpha = 0.05$) significance level, one would expect to incorrectly declare 5 QTLs. Using MAPMAKER/QTL under the generally accepted threshold value of 2.4 (Methods 1 and 2) does not take such issues into account.

Method 4 differs from Methods 1 to 3 by the population size used, which is 36% larger than that used in the other methods. The increased population size is a major advantage for Method 4, but prevents a direct comparison of method 4 to the other

methods is not applicable. However, analysis of the larger population by Method 4 provides further evidence verifying the QTL identified, since population size is a critical feature in one's ability to detect QTL (Darvasi et al. 1993). Because full RFLP data were not available for all the plants, the data from the full set had to be analyzed in a chromosomewise fashion. This was done using estimated threshold values derived from chromosomal analysis, which Churchill and Doerge (1994) refer to as chromosomewise threshold values. Method 4 could be of generalized value, since it provides considerable savings in time and cost for analysis of very large F_2 populations. In such a situation, one could use a random subset of the F_2 population for initial QTL mapping followed by use of the remainder of the F_2 plants for fine mapping and analysis by Method 4.

Comparison of these results to the regions detected within Solanum.

The maps of tomato and potato are very similar (Tanksley et al. 1992), and since some accession of the wild potato *Solanum berthaultii* produce low levels of acylsucroses (King et al. 1988), it is of interest to compare our results with those of Bonierbale et al. (1994), who examined regions associated with production of acylsucroses in populations derived from crosses of *Solanum berthaultii*. There is some concordance between the results of the QTL analysis in the two systems. Both mapping efforts found a broad region on chromosome 2, as well as a region on top of chromosome 4, to be associated with acylsugar levels. The differences between results of the two projects may be due to differences inherent in the two systems. Bonierbale et al. (1994) did not detect the region on chromosome 3 for levels of acylsugars, or the region at the bottom of chromosome 11 where we detect QTL activity for mole percent acylglucoses. However, if the region we detect on chromosome 11 chiefly affects levels of acylsugars through its effects on mole percent acylglucoses, it is not surprising that the region is not detected by Bonierbale et al. (1994), since *S. berthaultii* only produces acylsucroses.

We did not detect the region on chromosome 5 Bonierbale et al. (1994) have associated with trichome density and acylsugar levels. However, if that region has its effects on acylsugar levels through its effects on the density of acylsugar-secreting trichomes, it is not surprising that we would not detect the region, since this trait does not show much variation within our population. In addition, Bonierbale et al. (1994) shows a region toward the top of chromosome 11 for which we see a rise in LOD scores for our data on acylsugar levels, but the LODs never reached threshold values. Finally, we could not assay the top of chromosome 1 due to the homozygosity for that part of chromosome 1, but mapping in another small *L. esculentum* x *L. pennellii* F_2 population (60 plants) which segregated for markers in this region did not indicate the presence of regions associated with levels of acylsugars.

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Table 1. Accumulation of acylglucoses, acylsucroses and total acylsugars in F_2 and control populations

Population		Acylglucoses (nmoles)	Acylsucroses (nmoles)	Total Acylsugars (nmoles)
L. esculentum	average	39.8[z]	11.6	51.4
NY^{Lp4}	range	26.2 - 57.3[z]	8.8 - 16.8	35.0 - 74.1
F_1	average	115.2	133.0	248.2
	range	107.2 - 123.9	121.3 - 139.7	228.5 - 263.6
L. pennellii	average	1015.7	84.0	1099.7
LA716	range	859.2 - 1118.6	39.8 - 146.9	899.0 - 1265.5
F_2	average	76.3	116.8	482.3
popoulation	range	14.5 - 397.4	0 - 402.4	21.9 - 619.7

[z]The values included for the *L. esculentum* control show background due to the method rather than actual levels of acylsugars accumulated.

Table 2. QTL's identified for acylsucroses, acylglucoses, total acylsugars and mole percent acylgucoses using four analytical methods.

		Method 1[z]		Method 2, 3[z]			Method 4[z]			Summary		
QTL[y]	Trait[x]	LOD at peak[w]	% Var at peak[v]	LOD at peak[w]	% Var at peak[v]	Method 3 Threshold	LOD at peak[w]	% Var at peak[v]	Method 4 Threshold	Effect of Lp het.[u]	Effect of Lp homo.[u]	Gene action[t]
2A	ASUC	2.84	8.7	4.96	14.7	3.98	5.89	13.2	2.24	13.86 (20.79)	78.70 (77.00)	Le is partially dominant to Lp.
	TAS	2.55	7.8	3.73	11.3	3.55	4.91	11.1	2.18	34.15 (32.32)	110.04 (104.02)	Le is partially dominant to Lp.
	% AGLC	-	-	-	-	3.70	2.44	5.8	2.17	- (-6.00)	- (-16.31)	Le is partially dominant to Lp.
2B	ASUC	4.40	13.6	5.05	15.6	3.98	7.10	16.6	2.24	15.86 (21.37)	84.42 (86.16)	Le is partially dominant to Lp.
	TAS	3.53	10.7	4.31	12.9	3.55	6.58	14.7	2.18	18.96 (30.86)	109.72 (117.48)	Le is partially dominant to Lp.
	%AGLC	-	-	2.53	7.8	3.70	3.00	6.8	2.17	-4.54 (-8.31)	17.85 (-17.90)	Le is partially dominant to Lp.
3	ASUC	3.55	11.7	2.62	8.1	3.98	2.94	6.7	1.83	55.03 (41.56)	86.18 (68.08)	Lp is partially dominant to Le.
	TAS	3.97	12.3	3.14	9.8	3.55	3.44	7.9	1.86	93.31 (63.07)	139.83 (106.24)	Lp is partially dominant to Le.
	% AGLC	-	-	-	-	3.70	2.76	6.4	1.65	- (-19.08)	- (-14.39)	Lp is dominant to Le
4	ASUC	4.17	13.8	-	-	3.98	2.25	6.0	1.72	- (38.92)	- (2.77)	Heterosis?
	AGLC	-	-	2.41	7.4	4.34	4.13	9.2	1.72	19.55 (30.52)	57.08 (64.42)	Lp and Le co-dominant
	TAS	3.24	10.3	-	-	3.55	2.89	7.0	1.81	- (66.24)	- (66.84)	Lp is dominant, no additivity.
	% AGLC	-	-	2.41	9.2	3.70	1.87	4.8	1.77	-13.47 (7.12)	1.44 (5.18)	Situation unclear
11	ASUC	5.07	17.7	2.73	9.6	3.98	3.42	8.8	1.94	11.16 (7.98)	-47.00 (-37.44)	Le is partially dominant to Lp
	AGLC	2.54	8.4	-	-	4.34	2.86	7.2	2.03	- (25.62)	- (60.08)	Le is partially dominant to Lp
	% AGLC	5.20	17.8	6.57	22.2	3.70	7.17	17.7	1.83	7.47 (5.40)	30.30 (20.36)	Le is partially dominant to Lp

[z] Analytical methods 1 to 4 as described in Materials and Methods

[y] QTLs located: the proximal (2A) and distal QTLs on chromosome 2-lower, and the QTLs on chromsomes 3, 4, and 11.

[x] Traits: ASUC = acylsucroses, AGLC = acylglucoses, TAS = total acylsugars, % AGLC = mole percent acylglucoses

[w] Threshold for Methods 1 and 2 is 2.4, empirically derived thresholds for Methods 3 and 4 are presented in table.

[v] The percentage of the trait variance accounted for by the QTL.

[u] For effects of Lp heterozyous and homozygous conditions on each of the traits, the numbers presented are for Method 2,3 without (), and Method 4 with ().

[t] Allele action column presents conclusions on possible allele action based on the data in the effects of Lp het. and Lp homo. columns.

Fig. 1. Scatter plot of total acylsugars versus mole percent acylglucoses in the F_2 population.

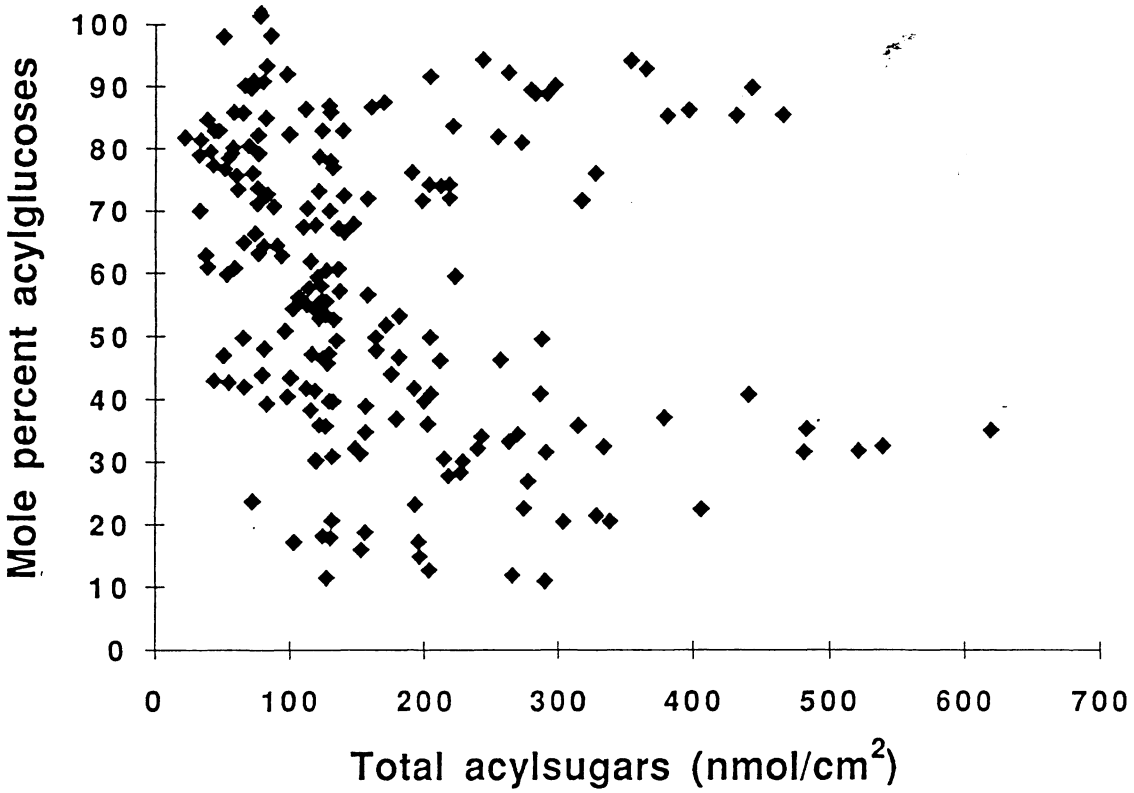
Fig. 2. Frequency distribution for percent *L. esculentum* genome of the F_2 population. The arrow indicated the mean and the horizontal line attached to the arrow indicates the 95% confidence interval of the mean.

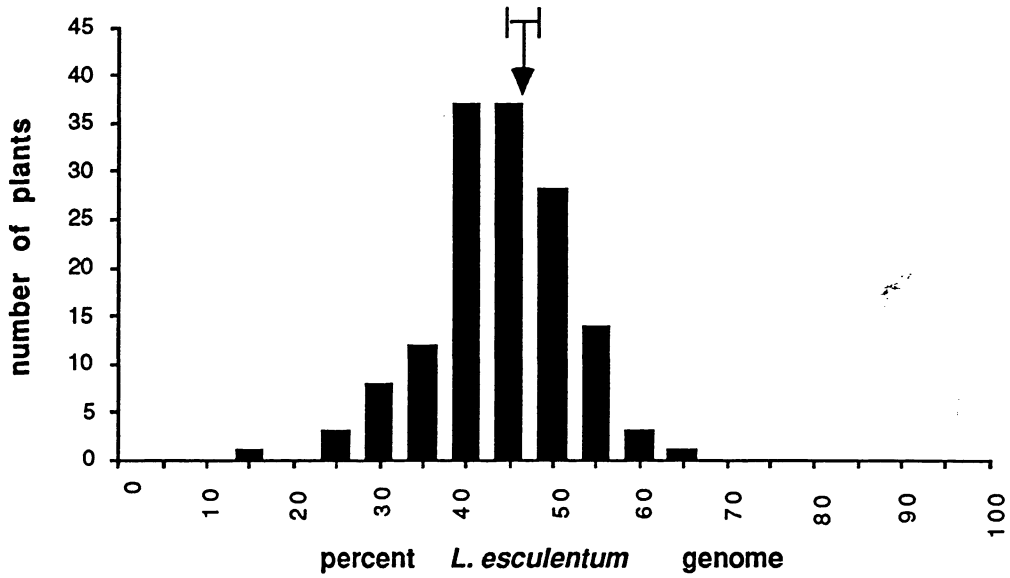
Fig. 3. Frequency distribution for the percentage of EE (%EE), EP (%EP) and PP (%PP) genotypes in the genome of the F_2 population. The arrows indicate the means and the horizontal lines attached to the arrows indicates the 95% confidence intervals of the means.

Fig. 4. Chromosome maps and locations of QTLs for acylsugar accumulation traits on chromosomes 2, 3, 4, and 11 (Fig 4a to 4d, respectively) using analytical Methods 1 to 4. Only the 2-lower portion of chromosome 2 is shown in 4a. Hollow bars parallel to the chromosomes indicate the 1 LOD range of a QTL and solid dots within these bars indicate the peaks of each QTL. Threshold values and LOD values at the peaks for each of the regions and methods are given in Table 2. Symbols used for the acylsugar traits are: ASUC = acylsucroses, AGLC = acylglucoses, TAS = total acylsugars, % AGLC = mole percent acylglucoses. A single bar indicates both Methods 2 and 3 (designated 2,3) in those cases that the LOD surpasses both thresholds. If no QTL is detected for a trait by a method on a particular chromosome, no heading and bar for that trait/Method combination is indicated on the figure. The proximal and distal QTLs identified on chromosome 2 are designated 2A and 2B at the right hand side of Figure 4a. The open ended bar with an arrowhead in figure 4a indicates that the bar in question extends to the end of the chromosome in the direction of the arrow. Open ended bars for Method 4 in Figures 4a to 4d

indicate that the 1 LOD range of the bar extends beyond that point, which is the end of the chromosome segment tested by this method.

as vs % glu plot Chart 2-1




number of plants
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
percent L. esculentum genome

=162

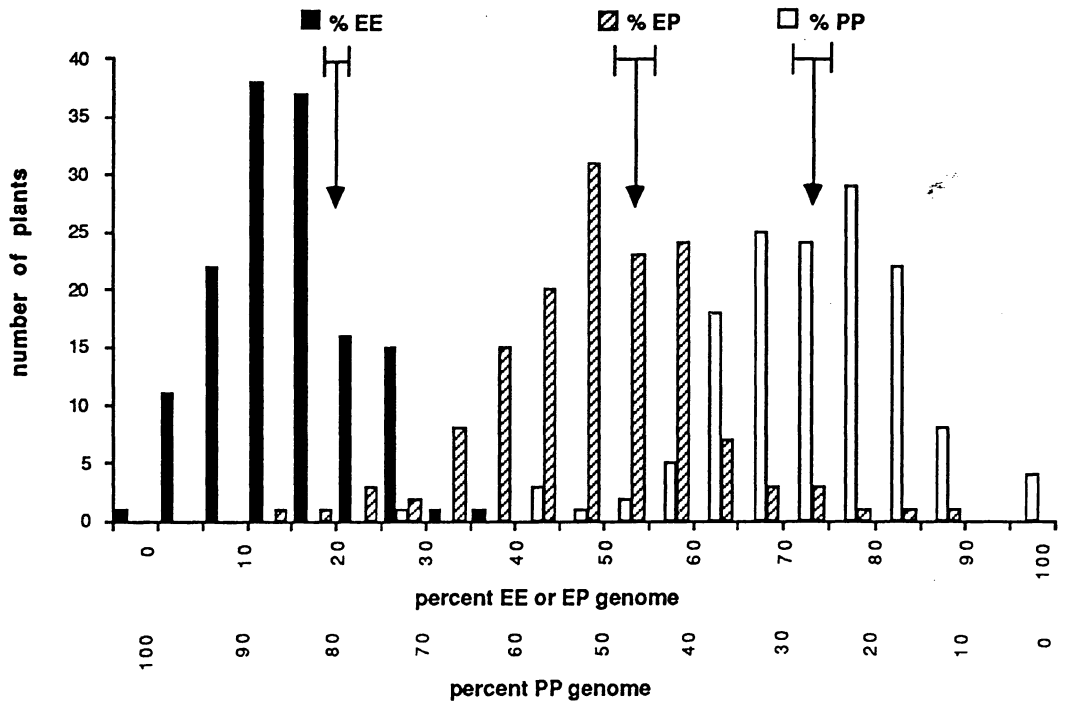
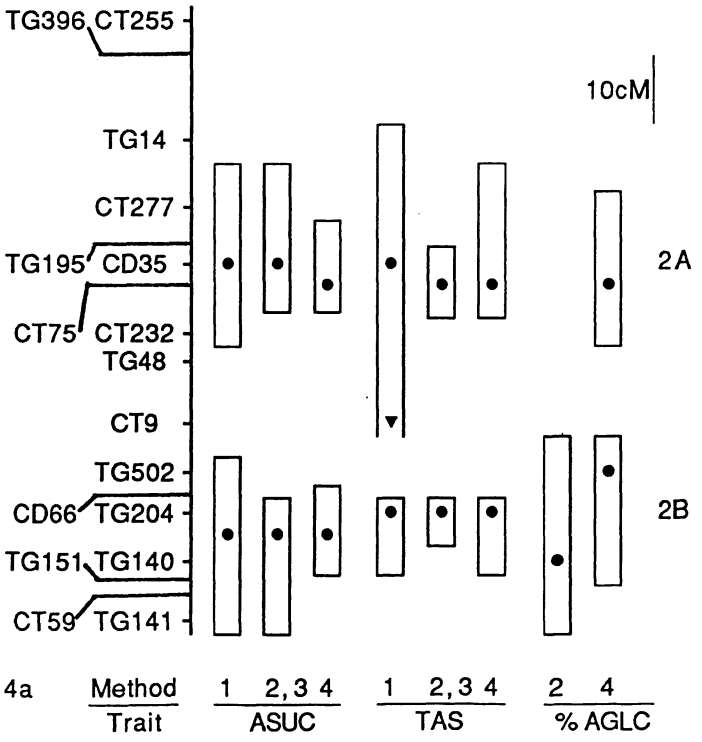

% EE
% EP
% PP
number of plants
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
percent EE or EP genome
100
90
80
70
60
50
40
30
20
10
0
percent PP genome

FIG 3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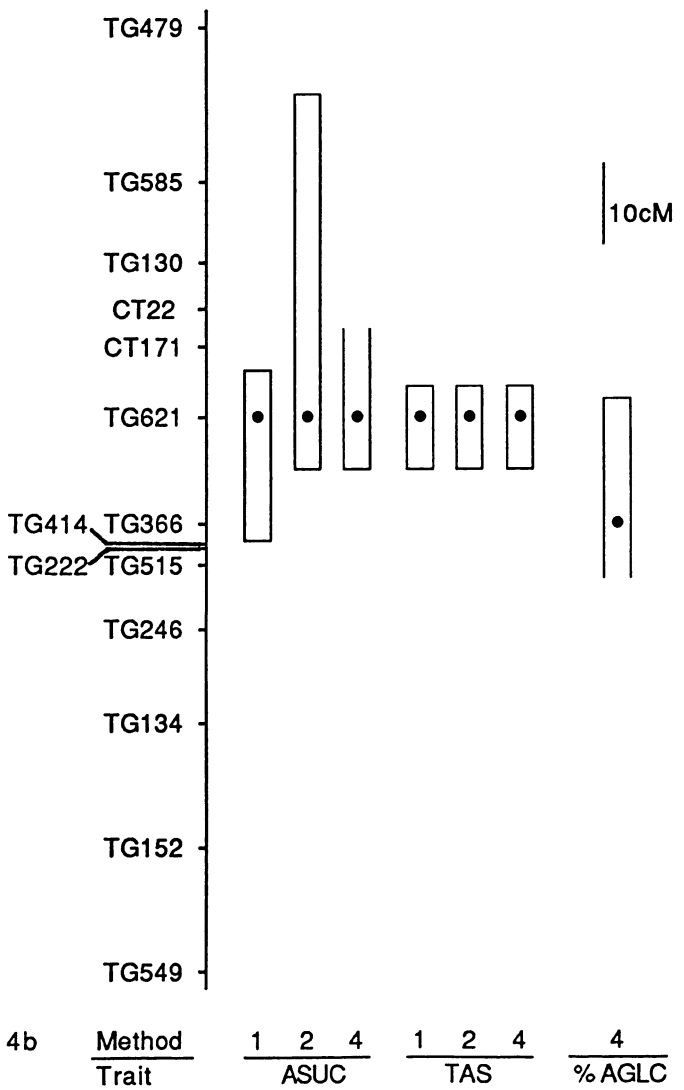
10cM
TG14
CT277
TG195
CD35
CT75
CT232
TG48
CT9
TG502
CD66
TG204
TG151
TG140
CT59
TG141
2A
2B
4a
Method
Trait
1 2,3 4
ASUC
1 2,3 4
TAS
2 4
% AGLC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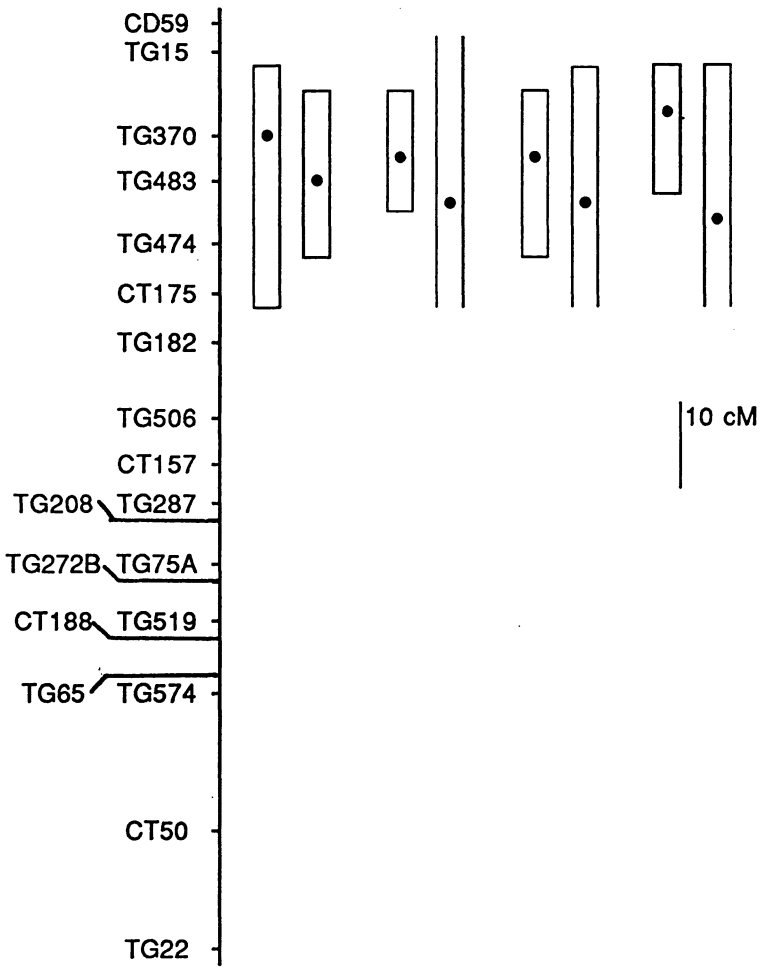
1 2 4
ASUC
1 2 4
TAS
4
% AGLC

4b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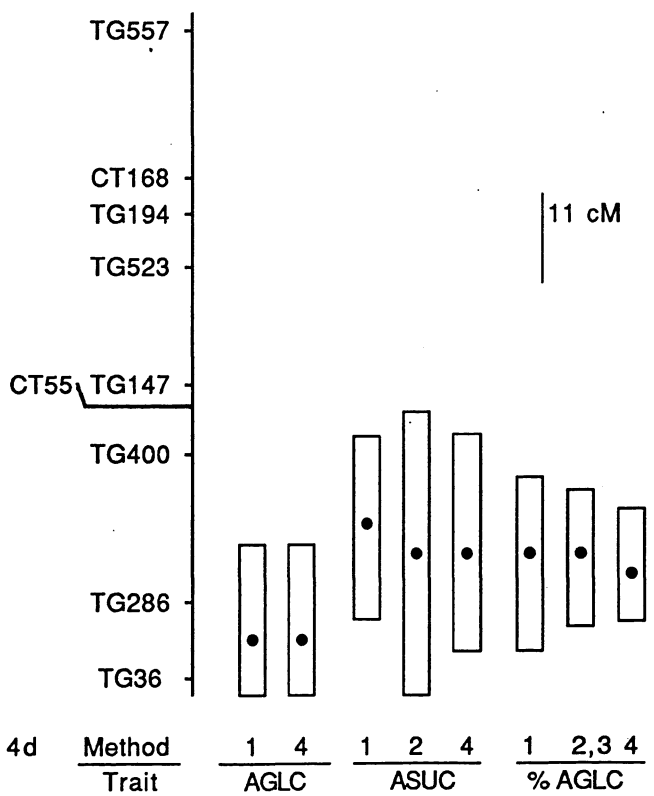
TG506
CT157
TG208
TG287
TG272B
TG75A
CT188
TG519
TG65
TG574
CT50
TG22
10 cM
Method
Trait
2 4
AGLC
1 4
ASUC
1 4
TAS
2 4
% AGLC

4c


TG557
CT168
TG194
TG523
CT55
TG147
TG400
TG286
TG36
11 cM
4d
Method
Trait
1 4
AGLC
1 2 4
ASUC
1 2,3 4
% AGLC